PHYSIOLOGICAL REGENERATION IN THE MUCOSA OF THE SMALL INTESTINE OF HYPOPHYSECTOMIZED RATS

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According to the results of numerous investigators, hypophysectomy produces inhibition of both normal and malignant growth in animals and has no effect, at least in certain animals, on the course of processes of regeneration and compensatory hypertrophy [5, 7, 9, 10, 16].

On the basis of the results in the literature it is difficult to assess the changes which hypophysectomy causes in physiological regeneration, a process which in the opinion of M. A. Vorontsova [1] lies at the root of processes of malignant growth, regeneration, and compensatory hypertrophy. It is known that hypophysectomy inhibits physiological regeneration in those organs whose function is regulated by one of the hypophyseal hormones, such as the gonadotrophins. As for the effect of hypophysectomy on physiological regeneration in organs whose function does not come under the direct regulating influence of hypophyseal hormones, information concerning this question is fragmentary and contradictory [14, 17]. Nevertheless, the solution of this question is of not only theoretical but also practical interest, since in recent years there have been published reports [15] of successful hypophysectomy in man, carried out in connection with repeated metastases of breast cancer. Hence a comprehensive study of the state of the hypophysectomized organism is important for clinicians [6, 3].

All the points mentioned above provided the grounds for carrying out the present investigation into physiological regeneration in the mucosa of the small intestine of hypophysectomized rats.

Physiological regeneration in the mucosa of the small intestine has frequently been the subject of investigations, as a result of which it has been established that the rebuilding phase of physiological regeneration is characterized by a certain level of mitotic activity, which regularly changes over the course of twenty-four hours [4, 8, 11, 13]. Although the results obtained by different investigators concerning the character of the daily rhythm in the mucosa of the small intestine are not always in agreement, the fact of its existence may nevertheless be considered proved. In the literature there are no results concerning the question of how the daily rhythm of mitotic activity in the mucosa of the small intestine changes after hypophysectomy. The only study is that of Leblond and Carriere [14], who devoted part of their paper to studies of the level of mitotic activity in the epithelium of the dudenal mucosa of hypophysectomized rats. During these investigations the authors observed that mitotic activity in the epithelium of the crypts of Lieberkühn in intact animals was 8%, and in hypophysectomized animals 3.9%; i.e., hypophysectomy leads to a two-fold decline in the number of mitoses.

The aim of the present study was to determine how the level and rhythm of mitotic activity in the intestinal mucosa changes after hypophysectomy.

METHODS

Male white rats weighing 150 g served as experimental animals. Between August 3 and August 11, 1959, animals were hypophysectomized by the method of Smith, with some modifications described in detail by S. G. Gasanov [2]. Experimental and control rats were kept, 5 or 6 to a cage, on a synthetic diet supplemented by wheat mash and milk. Between August 26 and August 27, 1959, the control and hypophysectomized animals were

sacrificed by decapitation at the following times: 10 A.M., 6 and 10 P.M., and 4 and 7 A.M.; 4-6 hypophysectomized and 8 control rats were sacrificed in each period. Completeness of hypophysectomy was evaluated on the basis of macroscopic observation of the excised hypophysis at the time of the operation, and was evaluated at the time of sacrifice of the animals on the basis of retarded growth and atrophy of the testes, as indicated by a decrease in body weight and weight of the testes of the experimental animals in comparison with the controls (see Table 1).

TABLE 1

Influence of Hypophysectomy on Body Weight, Weight of Testes, and Number of Cells in the Crypts of Lieberkühn in the Small Intestine of Rats

Group of animals	Body wt., g	Weight of testes, mg	No. of epithelial cells in a crypt
Final			
Control	209	1200	73.1
Experiment	144	365	53.4

For experimental purposes, only those animals were accepted in which the hypophysis was completely removed. Since hypophysectomies were carried out over the course of a week, while the animals were sacrificed within 24 hr, the period between hypophysectomy and death was not constant. In order that this difference might not be significantly reflected in the experimental results, animals were chosen for each period of sacrifice in such a way that the time from the moment of hypophysectomy was on the average the same for all groups of animals (19 days). Moreover, the experimental results were analyzed with respect to the individual length of life of an animal after hypophysectomy, and no corresponding differences were observed.

To study mitotic activity, pieces of small intestine taken just at the point of junction with the mesocolon were fixed in Bouin's fluid. Transverse paraffin sections 7μ in thickness were stained by hematoxylin and eosin. A count of mitoses at each phase was carried out in 50 crypts sectioned longitudinally. Mitotic activity was expressed as the number of mitoses per 1000 cells. For this purpose a count was taken of the number of cells in 200 crypts of control animals and 200 crypts of experimental animals. This count became essential because inspection of the preparations drew attention to the fact that crypts were shorter in hypophysectomized animals than in control rats. When the number of epithelial cells in one crypt was counted, this observation was borne out (see Table 1). In hypophysectomized rats each crypt contained on the average 53.4 cells, while this figure was 73.1 for control animals. The difference is statistically significant ($P = 0.001$).

TABLE 2

Diurnal Variations in Mitotic Activity in the Epithelium of the Crypts of Lieberkühn in Hypophysectomized and Control Rats

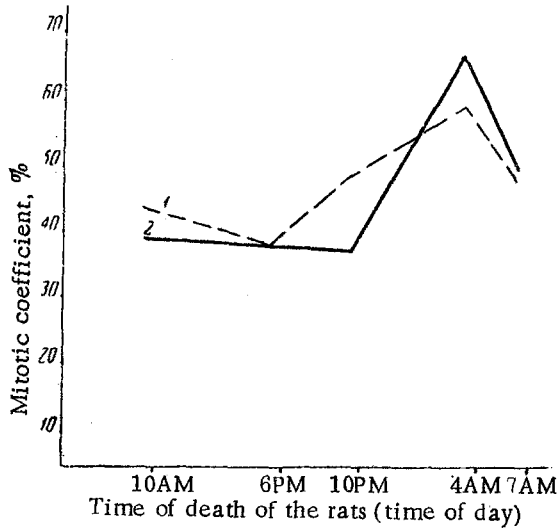
Fixation (time of day)	Number of mitoses occurring			
	in one crypt		per 1000 cells	
	control	expt.	control	expt.
10 A.M.	3,3	2,2	45	42
6 P.M.	3,1	2,2	41	41
10 P.M.	3,9	2,1	52	40
4 A.M.	4,6	3,7	62	68
7 A.M.	3,8	2,6	52	49
Average . .	3,7	2,5	50	48
	P=0,017		P=0,6	

In the present investigation, the difference in the number of cells in crypts of hypophysectomized and normal animals was taken into account in computing the mitotic coefficient. All the results were treated statistically by the Fisher-Student method.

RESULTS

The results of counting the number of mitoses in the epithelium of the crypts of Lieberkühn from the intestine of control and hypophysectomized rats are given in Table 2 and depicted graphically in the figure.

It is apparent from Table 2 and the figure that mitotic activity in the epithelium of the intestinal mucosa of control rats is at a low and approximately constant level at 10 A. M. and 6 P. M. At 10 P.M. an increase in mitotic activity begins to appear, but the increased number of dividing cells is not statistically significant ($P = 0.04$). The largest number of mitoses was detected at 4 A.M., and this declined somewhat at 7 A.M. The difference in the number of mitoses observed at 4 A.M. and 6 P.M. is statistically significant ($P = 0.001$). Differences among the number of mitoses observed at 4 A.M., 7 A.M., and 10 P.M. are not significant ($P = 0.06$). Analysis of the results by mitotic phases showed that the number of all phases of mitosis increased at 4 A.M.



Diurnal variations in mitotic activity in the epithelium of the crypts of Lieberkühn in the small intestine of hypophysectomized and control rats. 1) Control; 2) experimental.

It is also evident from the results in Table 2 that the average number of mitoses in one crypt of hypophysectomized animals is lower than the value in control rats by about 1/3. This difference is statistically significant. However, when the number of mitoses is computed on the basis of 1000 cells, taking into account the fact that the number of cells in the crypt of a hypophysectomized animal is lower than the control value by about 1/3 (see Table 1), this difference disappears. The average number of mitoses per 1000 cells in hypophysectomized animals was 48, and this value was 50 in controls; the difference is not statistically significant ($P = 0.6$). Consequently, it was not possible to detect any decline in mitotic activity in the mucosa of the small intestine of hypophysectomized rats. Moreover, it was observed that not only is there no change in the level of mitotic activity after hypophysectomy, but also the daily rhythm of cellular division persists in the epithelium of the crypts of Lieberkühn of hypophysectomized rats. It is evident from the results of Table 2 and the figure that the number of mitoses at 10 A.M. and 6 P.M. in hypophysectomized rats and also in controls is at the same rather low level. Unlike the controls, the hypophysectomized animals also have low mitotic activity at 10 P.M., but this increases sharply at 4 A.M. without the gradual transition observed in control rats.

At 7 A.M. some decrease in the number of mitoses takes place in both hypophysectomized and control rats, but the level of mitotic activity still remains high. The increased number of mitoses at 4 A.M. in experimental animals, as well as the decline at 7 A.M., is statistically significant ($P = 0.003$). Analysis of the results by mitotic phases showed that all phases of mitosis are involved in the increased number of mitoses at 4 A.M. in both experimental and control rats.

Thus, the results allow one to draw the conclusion that the rebuilding phase of physiological regeneration in the mucosa of the small intestine of control rats is characterized by a definite level of mitotic activity representing on the average 51 mitoses per 1000 cells, and by the presence of a diurnal rhythm of cellular multiplication; furthermore, the curve of the diurnal rhythm has a single-peak character, with maximum number of mitoses at 4 A.M. The maximal number of cellular divisions depends upon increased counts of all phases of mitosis. These results support the findings of some other investigators [8, 11, 13] as to the existence of a diurnal rhythm of cellular multiplication in the epithelium of the small intestine, and are in almost complete agreement with the experimental results of M. T. Gololobova [4].

As the results of our experiments showed, some atrophy of the mucosa of the small intestine sets in after hypophysectomy and is reflected, in particular, in the shortened length of the crypts of Lieberkühn and in the decreased number of epithelial cells in the crypts.

The observations completely support the results of Haeger, Jacobsohn, and Kahlson [12], who showed that the weight of the intestinal mucosa of cats declines by a factor of two within two weeks of hypophysectomy and that the fibers are significantly shortened.

From the results of the present study, hypophysectomy produces no changes in the level of mitotic activity, its diurnal rhythm, and ratios of mitotic phases in the mucosa of the small intestine of rats.

The discrepancy between these results and those of Leblond and Carriere [14] are apparently due to the fact that the latter authors, when analyzing their material, took no account of the difference in the length of the crypts and correspondingly in the number of nuclei per crypt in control and hypophysectomized animals.

The fact that the rebuilding phase of physiological regeneration is unchanged after hypophysectomy is important for appraisal of the over-all conditions of the hypophysectomized organism, inasmuch as one of the most important functions of the organism, digestion, is directly dependent upon the rate of cellular multiplication and the turn-over rate of epithelial cells of the crypts of Lieberkühn in the intestine.

SUMMARY

The number of mitoses was studied in the epithelium of Lieberkühn's crypts in control and hypophysectomized rats sacrificed at different times of the day. Hypophysectomy provoked no changes of the mitotic activity (as estimated per 1000 cells) nor of the diurnal rhythm of cell division. However, the number of cells in the crypts of the small intestine proved to be diminished in hypophysectomized animals.

LITERATURE CITED

1. Vorontsova, M. A., Regeneration of Lost Organs in Animals and Man [in Russian] (Moscow, 1953).
2. Gasanov, S. G., Probl. endokrinol. i gormonoter. 5, 5, 40 (1959).
3. Gluzman, F. A., Vrach. Delo, No. 2, p. 165 (1959).
4. Gololobova, M. T., Byull. Éksptl. Biol. i Med., No. 9, p. 118 (1958).
5. Kabak, Ya. M., Studies in the Dynamics of Development [in Russian] (Moscow, 1931) Vol. 6, p. 11.
6. Kandel', E. I., Voprosy Neirokhir., No. 5, p. 56 (1959).
7. Astarabadi, T. M., Essex, H. E. and Grindlay, J. H., Endocrinology 52, 652 (1953).
8. Blumenfeld, C. M., Arch. Path. 35, 667 (1943).
9. Braun-Menendez, E. and Houssay, H. E. J., C. R. Soc. Biol. 143, 1255 (1949).
10. Brolin, S. E. and Hellman, B., Acta path. microbiol. scand. 38, 433 (1956).
11. Bullough, W. S., Proc. Roy. Soc. B. 135, 879, 212 (1948).
12. Haeger, K., Jacobsohn, D. and Kahlson, G., Acta physiol. scand. 30, 111, p. 161 (1953).
13. Klein, H. and Geisel, H., Klin. Wschr. 24-25, 626 (1947).
14. Leblond, C. P. and Carriere, R., Endocrinology 56, 261 (1955).
15. Luft, R. and Olivecrona, H., Neurosurgery 10, 301 (1953).
16. Rolt, D. and White, H. L., Endocrinology 53, 436 (1953).
17. Teir, H. and Carpén, I., Acta path. microbiol. scand. 47, 291 (1959).

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